

Standard Operating Procedure for Chlorophyll *a* Sampling Method Field Procedure

LG404

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Standard Operating Procedure for Chlorophyll a Sampling Method Field Procedure

1.0 SCOPE AND APPLICATION

- 1.1 This method is used to sample chlorophyll-containing algae from the Great Lakes and Tributary streams.

2.0 SUMMARY OF METHOD

- 2.1 A representative lake water sample is collected from Niskin bottles from various depths and filtered by vacuum filtration in dim light. The filter is then placed in a screw cap culture tube which is stored in the dark at sub-freezing temperatures and shipped to the laboratory for extraction and analysis.

3.0 APPARATUS

300-mL plastic filter funnel with magnetic base, Gelman
Vacuum system (3-4 psi)
GF/F filters, Whatman (47-mm)
16 x 100 mm disposable glass screw-cap culture tubes
Pasteur short disposable pipettes
Rubber bulb
Plastic wash bottle, 500-mL
Plastic wash bottle with eye dropper, 500-mL, for MgCO_3
Filter forceps
Opaque sample bottles, 1000-mL (Nalgene or equivalent)

4.0 REAGENTS

- 4.1 **Saturated Magnesium Carbonate Solution:** Ten grams of magnesium carbonate are added to 1000 mL of reagent water. The solution is settled for a minimum of 48 hours, after which the clear solution is decanted into a new container for subsequent use. **Only the clear "powder free" solution is used during subsequent steps.**

5.0 SAMPLE HANDLING AND PRESERVATION

- 5.1 The entire procedure is carried out as much as is possible in subdued light (green) to prevent photo-decomposition. The frozen samples are protected from light during storage for the same reason. During the filtration process, the samples are treated with a MgCO_3 solution (section 4.1) to eliminate acid induced transformation of chlorophyll to its degradation product, pheophytin. Samples are grouped by station and completely wrapped in aluminum foil during storage and transport to a land-based laboratory. The samples are transported to the laboratory in a cooler containing dry ice. Analysis is performed as soon as possible following sampling and always within three and a half weeks.

6.0 FIELD PROCEDURE

- 6.1 A list of all chlorophyll samples along with the corresponding preprinted labels, is prepared *before the start of each cruise*.
- 6.2 Opaque Nalgene bottles, permanently labeled with depth codes, are used to carry the water samples from the Rosette Niskin sampling operation to the ship's biology lab. Prior to each station, the appropriate bottles for that station are delivered to the sampling deck and the appropriate labels affixed to the culture tubes. The labels are covered with clear plastic tape to protect against wetness.
- 6.3 After filling, the opaque bottles are held in the biology lab refrigerator for up to an hour before filtering. Samples should be filtered within 30 minutes, but may sit up to 2 hours in refrigeration, only under unusual circumstances and must be noted on data sheets.
- 6.4 Filter forceps are used to place 47-mm diameter Whatman GF/F filters, textured side up, on Gelman magnetic filter funnels in a filtration manifold.
- 6.5 Sample Volume:

Due to differing trophic levels among the Great Lakes, the volume of water filtered varies. For Lake Erie, 150 mL of sample is filtered. For Lakes Ontario, Huron, Michigan, and Superior, a 250-mL sample is used. As each sample is filtered, the volume filtered is entered on a spreadsheet.
- 6.6 A vacuum pressure of no more than 5 psi (10 in Hg, or 2.5 cm Hg) is used.
- 6.7 The sample bottle is inverted several times to create a uniform mixture, and the 250-mL graduate is rinsed 3 times with sample water prior to measuring the sample volume. The sample volume is measured from the bottom of the meniscus.
- 6.8 The sample volume is added to the filter funnel, the valve of the filtration unit is turned on and the graduate is rinsed with 10 to 20 mL of reagent water which is added to the filter funnel.
- 6.9 When 10-50 mL of sample remains on the filter, 10 drops of the MgCO_3 solution are added, using a disposable pipette. The sides of the filter funnel are rinsed with reagent water. The valve on the filtration unit is switched off as soon as the liquid disappears, to prevent the breakage of cells.
- 6.10 With forceps, the filter is carefully removed from the funnel folded in half, rolled, and placed into the bottom of the pre-labeled culture tube which is then tightly closed.
- 6.11 When all samples for a station have been filtered, the tubes are wrapped as a group in aluminum foil. Using masking tape, the aluminum foil package is labeled with the lake, station and date, and the package is immediately placed in the freezer. All of the above procedures are completed in subdued (green) light.

7.0 QUALITY ASSURANCE

- 7.1 Each of the following audits is collected once per lake basis (approximately 20 samples).
- 7.2 Field duplicates are taken from a second Niskin bottle closed at about the same time and location as the regular field sample. It is transported from the Niskin bottle to the onboard biology laboratory in an opaque bottle marked as duplicate sample.
- 7.3 Laboratory duplicates are filtered from the same opaque sample bottle as their corresponding regular field samples.
- 7.4 Field blanks (Field Blk), consisting of reagent water are carried by an opaque sample bottle from the onboard Barnstead reagent water system to the filtration apparatus. The bottle is used only for field blanks and is permanently marked as such.

8.0 SAFETY AND WASTE HANDLING

- 8.1 Refer to GLNPO's *Health, Safety and Environmental Compliance Manual* (May 1997, or as amended) and individual instrument procedural operations manuals for specific details on applicable 1) personal health and safety issues; 2) instrumental, chemical, and waste handling procedures; and 3) accident prevention. This applies to all EPA personnel, EPA contractors or federal, state, or local government agencies, and persons who operate or are passengers onboard US EPA GLNPO vessels during all activities and surveys.
- 8.2 All applicable safety and waste handling rules are to be followed. These include proper labeling and disposal of chemical wastes. Over-board discharges of chemical wastes are forbidden.
- 8.3 During sampling, caution, common sense, and good judgement should dictate appropriate safety gear to be worn in any given situation on deck. Hard hats, gloves, and steel-toed shoes must be worn in working conditions where there is a possibility of injury to the head, hands, or feet; however, if in doubt, please ask the Chemical Hygiene Officer.
- 8.4 Collecting samples in cold weather, especially around cold water bodies, carries the risk of hypothermia and frostbite. Sampling team members should wear adequate clothing for protection in cold weather. For specific information regarding sampling during cold conditions, please refer to the US EPA GLNPO *Standard Operating Procedures for Winter Operations* (December 1994, or as amended).
- 8.5 Collecting samples in extremely hot and humid weather carries the risk of dehydration and heat stroke. Sampling team members should carry an adequate supply of water or other liquids for protection against dehydration in hot weather.
- 8.6 Work vests must be worn while working on the fantail and Rosette deck.

9.0 SHIPPING

- 9.1 After about 35 samples have been collected, or at the conclusion of sampling in a lake, the available samples are wrapped into one complete batch and clearly labeled with survey, lake, and date. These samples are stored in the ships walk-in freezer until they can be transported to the land-based laboratory. For transport, the batches are packed in a cooler with adequate dry ice to last for the duration of the trip. Upon arrival at the land-based laboratory, the samples are placed in the freezer (-20°C) until they can be analyzed.